Published Inconsistencies in Methyl Red and Voges-Proskauer Reactions of *Klebsiella pneumoniae*

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Inconsistencies in the results of methyl red and Voges-Proskauer tests of *Klebsiella pneumoniae* have been published. These inconsistencies result from the unresolved taxonomic position of *K. aerogenes*.

In spite of publications of definitive works, the taxonomic differentiation between the genera *Klebsiella* and *Enterobacter* has remained unclear. Rapid and precise identification is necessary in both clinical and pollution microbiology, particularly in view of the apparently ubiquitous distribution of *Klebsiella* species in both oligotrophic and eutrophic systems.

In studying the occurrence of *Klebsiella pneumoniae* in the environment, we have discovered a basic discrepancy in the current literature relative to identification, specifically methyl red (MR) and Voges-Proskauer (VP) reactions. *K. pneumoniae* has been reported to be isolated much more frequently than other members of the genus (11), and recently its wide distribution in aquatic systems has been noted (18). Since it is probable that *K. pneumoniae* will gain prominence as an indicator organism, it becomes imperative for investigators to be able to recognize and identify it (Table 1).

Investigation into the current literature revealed that researchers found that high percentages of *K. pneumoniae* isolates were MR⁻ and VP⁺ (1, 9-11, 14-17). These isolates came from a variety of sources, including feces, clinical materials, sugar cane and other agricultural products, forest products, and eutrophic and oligotrophic waters. Burrows (4) stated that the coliform group was separated on the basis of the four biochemical tests: indole, methyl red, Voges-Proskauer, and citrate, represented by the mnemonic IMViC. Classically, *Escherichia coli* is represented by ++-- , the *Klebsiella-Enterobacter* group is represented by --++ , and an intermediate form, *Citrobacter freundii*, is represented by +-+ .

Cowan et al. (7) published a classification of *Klebsiella*, subdividing the genus into six categories, including pathogenic *K. pneumoniae* and saprophytic *K. aerogenes*. Duguid (8) reported that *K. aerogenes* was fimbriated whereas *K. pneumoniae* was not, further adding to the separation. In 1973, Fallon (12) reported *K. aerogenes* as the predominant species isolated from *Klebsiella*-contaminated sputum

samples, but he determined that it was seldom pathogenic. *K. pneumoniae* was isolated from only 2.3% of the sputum samples cultured, but better than half of those isolated were considered to be disease related. Fallon primarily utilized the taxonomic scheme of Cowan et al. (7) and Cowan and Steel (6) in identification of the isolates. Bascomb et al. (3) also supported the separation of *K. aerogenes* from *K. pneumoniae*.

Cowan (5) has recently published a revision of the 1960 taxonomic scheme, which reiterates the need for six species or groups instead of the three that Ørskov (17) designated in *Bergey's Manual*. Cowan designated one species *Klebsiella aerogenes*, which other workers consider to be synonymous with *K. pneumoniae* (10, 11, 17). His supporters have found that *K. aerogenes* is saprophytic, serologically falls into all types, and is MR⁻ and VP⁺. On the other hand, the *K. pneumoniae* (sensu stricto) of Cowan is pathogenic, serologically only type 3, and MR⁺ and VP⁻. Thus, the consolidation of the two species, as has been done by Ewing (11) and Ørskov (17), offers a degree of inconsistency in IMViC results.

Johnson et al. (13), in a recent work on numerical taxonomy of *Enterobacteriaceae*, used strains designated by other sources as *K. aerogenes* and *K. pneumoniae*. Results of IMViC and other distinguishing tests were inconclusive, and they classified both clusters under the single species *K. pneumoniae*.

With the advent of more sophisticated monitoring of aquatic systems, klebsiellae have become important both as indicator organisms and as potential water-borne pathogens (15, 18). Although clinical laboratories may not routinely use MR and VP in their determinations, aquatic microbiologists have found IMViC to be useful in confirmation of presence of fecal coliforms (2). The published inconsistencies in the IMViC results for *K. pneumoniae* are confusing, particularly since mouse virulence tests and serological typing are not done routinely in aquatic monitoring, and work recently pub-

TABLE 1. *IMViC reactions for Klebsiella pneumoniae and Klebsiella aerogenes as published between 1960 and 1974*

Reference	IMViC reactions for:	
	<i>K. pneumoniae</i>	<i>K. aerogenes</i> ^a
Cowan (5)	- + - +	- - + +
Johnson et al. (13)	- 4/11 + + ^b	- 3/52/5 +
Cowan et al. (7)	() ^c + - +	- - + +
Ewing (11)	- - + +	^c
Lennette et al. (14)	- - + +	^c
Ørskov (17)	(var) - + d	^c
Ales Reinlein et al. (1)	- - + +	^c
Burrows (4)	- - + +	^c

^a *K. aerogenes* is not considered a valid taxon by Ørskov (17) and other workers.

^b 4/11 indicates the number +/number tested.

^c Information not given.

lished indicates that aquatic-borne klebsiellae fitting Cowan's and Fallon's descriptions for *K. aerogenes* are pathogenic for mice (15). Although there is no argument regarding the fact that *K. aerogenes* is properly relegated to the genus, the difference in MR and VP results may lead to misidentification of aquatic isolates.

It is suggested that aquatic microbiologists associated with monitoring *Klebsiella* species utilize other tests, particularly ornithine decarboxylation and motility, for identification of suspected isolates. This should reduce the possibility of misidentification until the taxonomic question can be resolved.

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REPRINT REQUESTS

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